c-*myc* and pRB: Role in TGF-β1 Inhibition of Keratinocyte Proliferation

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Transforming growth factor β1 (TGF-β1) is the prototype of a large family of growth factors involved in cell growth control, development, angiogenesis, and wound healing (for review, see Barnard et al. 1990). This polypeptide growth factor displays a wide array of biological activities that vary depending on the cell type involved. TGF-β1 stimulates extracellular matrix formation and fibroblast growth and migration in vitro, events critical for wound healing (Moses et al. 1985; Shipley et al. 1985; Ignotz and Massagué 1986; Ignotz et al. 1987; Raghow et al. 1987). Stimulation of cell growth appears to be indirect, mediated through stimulation of platelet-derived growth factor production (Leof et al. 1986). Proliferation of most normal cell types in culture is inhibited by TGF-β1. In vivo, TGF-β1 has been shown to inhibit proliferation of mammary epithelial cells, hepatocytes, lymphoid cells, and bone marrow stem cells (for review, see Moses et al. 1990).

Previous studies have linked TGF-β1 inhibition of skin keratinocyte growth to suppression of the c-*myc* proto-oncogene (Coffey et al. 1988). TGF-β1 markedly decreased the level of c-*myc* mRNA in rapidly growing BALB/MK mouse keratinocytes, and protein synthesis was required for this effect. In BALB/MK cells made quiescent by epidermal growth factor (EGF) depletion and subsequently stimulated by addition of EGF, DNA synthesis can be prevented by the addition of TGF-β1 at any point up to the G_1/S boundary (Pietenpol et al. 1990a). Similar results have been reported for mink lung (Mv1Lu) cells (Laiho et al. 1990; Howe et al. 1991). However, since c-*myc* expression is induced rapidly and remains elevated throughout G_1 into early S phase (Coffey et al. 1988 and unpubl.), c-*myc* is a candidate for mediation of TGF-β1 growth inhibition. Because c-*myc* has been shown to be essential for proliferation of other cell types (Holt et al. 1988), we proposed that c-*myc* may be a reasonable target for the inhibitory actions of TGF-β1 in the keratinocytes. This hypothesis was supported by the finding that c-*myc* antisense oligonucleotides which inhibit synthesis of Myc protein reduced proliferation of BALB/MK cells, indicating that c-*myc* expression is probably also required for keratinocyte growth (Pietenpol et al. 1990a). Further studies showed that TGF-β1 blocked transcriptional initiation of the c-*myc* gene and that a region spanning from −86 to −63 relative to the P1 transcription start site, termed the *TGF-β control element* (TCE), is necessary for TGF-β1 inhibition (Pietenpol et al. 1990a, 1991).

Recently, it was found that TGF-β1 failed to inhibit cell growth of human keratinocytes immortalized by human papillomavirus (HPV)-16, HPV-18, or SV40 (Pietenpol et al. 1990b). In addition, c-*myc* mRNA expression was not affected by TGF-β1 in cells containing these oncoproteins. As stated above, expression of c-*myc*-CAT (chloramphenicol acetyltransferase) reporter plasmids in BALB/MK cells or primary human keratinocyte (HFK) cultures was markedly inhibited by addition of TGF-β1. However, when these cells were cotransfected with constructs encoding viral oncoproteins that bind retinoblastoma gene product (pRB) (HPV-16 E7, SV40 T antigen, or adenovirus type 5 E1A) and the c-*myc*-CAT constructs, CAT expression was not suppressed by TGF-β1 treatment. Transfection of c-*myc*-CAT with constructs expressing the DNA tumor oncoproteins mutated in the pRB-binding domain failed to block inhibition by TGF-β1, suggesting that pRB may be involved in the TGF-β1 inhibition of c-*myc* expression and of cell growth. Further evidence to support a role of pRB comes from experiments demonstrating repression of c-*myc*-CAT either by TGF-β1 or by cotransfection with a pRB expression vector (Pietenpol et al. 1991). The TCE of the c-*myc* promoter that is necessary for TGF-β1 suppression was also found to be required for inhibition by cotransfection with pRB expression plasmids (Pietenpol et al. 1991).

In the present study, we show that proliferation and c-*myc* expression in the DU145 human prostate adenocarcinoma cell line, which lacks normal pRB but contains TGF-β1 receptors, are not inhibited by TGF-β1. This result provides further evidence that pRB is a necessary component in the pathway for TGF-β1 suppression of c-*myc*. Binding studies with oligonucleotides containing the TCE have revealed additional cellular protein(s) in the TGF-β1 pathway for inhibition of c-*myc* transcription and proliferation.

METHODS

Cell culture. Murine BALB/MK cells were passaged in low-calcium minimal essential medium (MEM) supplemented with 4 ng/ml EGF and 8% dialyzed fetal calf serum (FCS) (Weissman and Aaronson

1983). Cells were used within ten passages after recovery from frozen stocks. Primary cells from human epidermal keratinocytes were isolated from newborn foreskin and grown in serum-free MCDB 153 complete medium (Wille et al. 1984). SV40-transformed HFK (SV40/HFK) cells were grown in media consisting of three parts MCDB 153 complete medium plus one part DMEM media with 10% FCS. DU145 and PC3 prostate carcinoma cell lines were obtained from American Type Culture Collection and cultured in McCoy's 5A media supplemented with 10% and 5% FCS, respectively. Retinoblastoma Y79 cells were cultured in RPMI media with 20% FCS. All terminal cultures used in experiments were grown in the presence of 100 units/ml penicillin/100 ng/ml streptomycin. Cells were routinely tested and found to be free of mycoplasma (Chen 1977).

Cell growth experiments. Cells were plated in normal growth media at 4×10^4 cells per well into 24-well plates. After 24 hours, TGF-β1 was added to the media at the appropriate concentrations (0.01–10.0 ng/ml). Cells were labeled with 1 μCi/ml [^{3}H]thymidine for 2 hours, 22–24 hours after the addition of TGF-β1. Cells were rinsed twice with phosphate-buffered saline (PBS), precipitated with 10% TCA, and resuspended in 300 μl/well of 0.2 N sodium hydroxide. Samples (100-μl aliquots) were counted in a scintillation counter.

Northern analysis. Isolation and electrophoresis of total cell poly(A)$^+$ RNA have been discussed previously (Coffey et al. 1988). Briefly, DU145 cells were grown in normal growth media in 150-cm^2 flasks until approximately 70% confluent. Cells were treated with media containing 10 ng/ml TGF-β1 for 0, 0.5, 1, 3, 6, 12, or 24 hours and harvested in lysis buffer for Northern analysis. Cell lysates were treated with proteinase K, and poly(A)$^+$ RNA was selected by running samples over oligo(dT) columns. Samples (3 μg of poly(A)$^+$ RNA/lane) were loaded and run on a 1% formaldehyde/agarose gel. RNA was transferred onto a Nitroplus nitrocellulose filter, and the blot was probed with a ^{32}P-end-labeled c-*myc* probe. The probe (p1B15) to the constitutively expressed cyclophilin gene was used to control for gel loading (Danielson et al. 1988).

Protein/DNA (Southwestern) analysis. Cells were cultured in 100-mm plates under the indicated experimental conditions. The cell monolayers were washed twice with 10 ml of 4°C PBS. Lysis buffer (250 mM NaCl, 0.1% Nonidet P-40, 50 mM HEPES at pH 7.0, 25 μm of sodium fluoride; 200 μM sodium orthovanadate, 0.12 TIU aprotinin) was added to the monolayers (1.0 ml/plate), and the plates were rocked on ice at 4°C for 30 minutes. Cells were scraped with a rubber policeman, and the cell suspension was centrifuged at 15,000 rpm for 5 minutes. The supernatant was removed from the pelleted cellular debris, and total cellular protein extract (100 μg) was diluted 1:1 with sample resuspension buffer (0.125 M Tris-HCl at pH 6.8, 10% β-mercaptoethanol, 4% SDS, 20% glycerol). Samples were boiled for 5 minutes and loaded onto a 7.5% SDS-polyacrylamide gel in a buffer of 25 mM Tris-HCl (pH 8.3), 192 mM glycine, and 0.1% SDS (Laemmli 1970). Electrophoresis was carried out at 40 V. The gel was blotted to nitrocellulose (0.45 μm) at room temperature on a Bio-Rad transfer cell at 40 V for 2 hours in gel-transfer buffer (12.5 mM Tris-HCl, 100 mM glycine at pH 8.3). Transfer of proteins was monitored by use of prestained high-molecular-weight standards. The nitrocellulose was blocked overnight at 4°C in 50 ml of 5% Carnation nonfat dry milk, 0.1% Tween 20, and 1 mM dithiothreitol (DTT) in 1 × TNE (25 mM Tris at pH 7.5, 50 mM NaCl, 2.5 mM EDTA). Following blocking, the nitrocellulose was washed in 1 × TNED (10 mM Tris at pH 7.5, 50 mM NaCl, 0.1 mM EDTA, 1 mM DTT) two times for a total of 15 minutes and then placed in a sealed plastic bag containing 20 ml of 1 × TNED, 10 μg/ml poly(dI-dC)·(dI-dC), and 1×10^6 cpm/ml ^{32}P-labeled TCE or TCE.m oligonucleotide probe. Binding was performed for 1 hour at room temperature with gentle agitation. The nitrocellulose was washed three times (10 min each) with 1 × TNED at room temperature. The filter was allowed to air dry at room temperature and exposed to Kodak X-Omat AR film (12–24 hr) with an intensifying screen at −70°C.

RESULTS

Effect of TGF-β1 on Proliferation and c-*myc* Expression in pRB-defective Cells

If pRB is involved in the TGF-β-mediated suppression of c-*myc* expression and growth inhibition as suggested by previous studies, cells with a deleted or defective *RB* gene should be insensitive to TGF-β1. Retinoblastoma cells are not inhibited by TGF-β1; however, these cells lack TGF-β receptors (Kimchi et al. 1988). The DU145 prostate adenocarcinoma line contains a mutated *RB* gene and expresses a nonfunctional, truncated form of pRB (Bookstein et al. 1990). This cell line has TGF-β receptors as determined by competitive binding assays (Wilding et al. 1989) and affinity cross-linking (J.A. Pietenpol and H.L. Moses, unpubl.). The response of this line to inhibition by TGF-β1 was therefore examined. Unlike TGF-β1-sensitive BALB/MK, HFK, or Mv1Lu mink lung cells (data not shown) (Shipley et al. 1986; Coffey et al. 1988; Pietenpol et al. 1990b), the growth of DU145 cells was not affected by treatment with TGF-β1 at concentrations ranging from 0.01 to 10.0 ng/ml (Fig. 1A). In addition, the levels of endogenous c-*myc* mRNA in these cells were not decreased by treatment with TGF-β1 at 10.0 ng/ml over a 24-hour period (Fig. 1B). These results are consistent with the hypothesis that pRB is necessary for TGF-β1 suppression of c-*myc* transcription and growth inhibition.

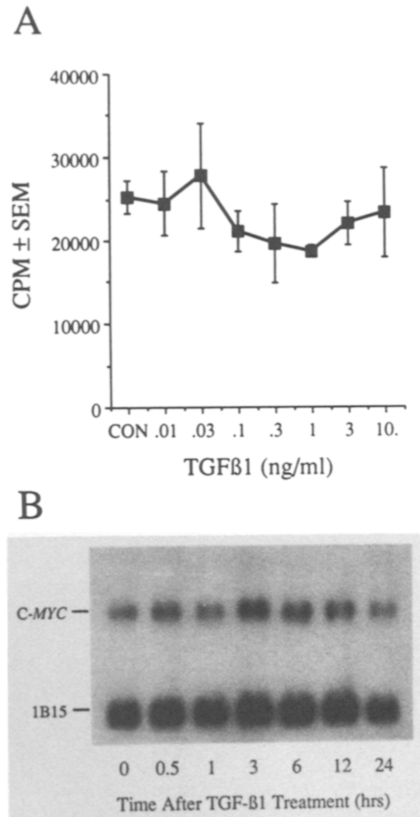

Figure 1. Response of TGF-β1-insensitive DU145 prostate adenocarcinoma cells to TGF-β1 treatment. (*A*) Amount of [^{3}H]thymidine incorporation after TGF-β1 treatment of DU145 cells is shown. Cells were treated for 24 hr with appropriate concentrations of TGF-β1 and pulsed with [^{3}H]thymidine 22–24 hr after TGF-β1 addition. Each point represents an average of counts from three wells, plus or minus standard error of the mean (S.E.M.). (*B*) Northern blot analysis of c-*myc* mRNA from DU145 cells treated with TGF-β1 (10 ng/ml) for times indicated. Constitutive probe 1B15 (Danielson et al. 1988) is used to control for differences in gel loading.

TCE Binding to Cellular Proteins

Since previous studies indicated that pRB is necessary for TGF-β1 suppression of *myc* transcription, we wished to determine whether pRB or other cellular factors bind directly to the TCE in the c-*myc* 5′ regulatory region. Expression of c-*myc*-CAT constructs containing the intact TCE in both HFK and BALB/MK cells is significantly decreased by treatment of the cells with TGF-β1 or by cotransfection with pRB expression vectors (Pietenpol et al. 1990a, 1991). The specificity of the TCE for this effect was demonstrated in experiments which showed that expression of constructs containing a mutated TCE promoter region (Fig. 2) was not inhibited by either TGF-β1 or cotransfection with pRB (Pietenpol et al. 1991).

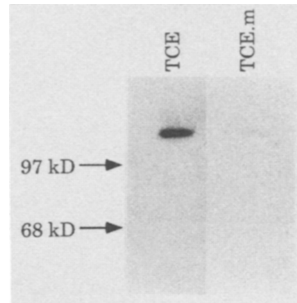

Figure 3. Specific binding of TCE to cellular proteins. Cellular proteins were isolated from rapidly growing HFK cells, separated by 7.5% SDS-PAGE, and transferred to nitrocellulose filters. The filters containing 100 μg of protein/lane were incubated individually with 2.9×10^7 cpm ^{32}P-labeled oligonucleotide hybrids representing the wild-type TCE or the mutant TCE (TCE.m).

Binding of TCE and TCE.m oligonucleotide probes to human keratinocyte cellular extracts was determined. Proteins from human keratinocytes were separated by SDS-PAGE and blotted to nitrocellulose, and the blots were probed with either TCE or TCE.m ^{32}P-labeled oligonucleotides (Fig. 3). The TCE probe bound a protein of approximately 106 kD (termed p106). Binding was carried out in the presence of a 400-fold excess of nonspecific DNA. Furthermore, TCE.m did not bind to p106, suggesting that the TCE-p106 interaction is specific. Similar binding results were obtained using extracts from mouse keratinocytes (data not shown).

To determine whether the binding of TCE to p106 was regulated by TGF-β1 treatment, rapidly growing BALB/MK cells were treated for various times with TGF-β1 (10 ng/ml), and proteins were separated by SDS-PAGE, blotted, and probed with TCE. TCE binding to p106 was significantly decreased at 2 and 4 hours of TGF-β1 treatment, with recovery of binding at 12 and 24 hours (data not shown). Further analysis revealed that TCE binding to p106 was significantly reduced within 10 minutes of treatment, with a maximal inhibition of 65% occurring at 240 minutes (Fig. 4). Similar results showing TGF-β1 inhibition of TCE-p106 binding were obtained in HFK cells (data not shown).

SV40/HFK cells are resistant to TGF-β1 inhibition of cell growth, and levels of c-*myc* mRNA in these cells are not affected by TGF-β1 (Pietenpol et al. 1990b). Therefore, Southwestern analyses of protein extracts

TCE $^{-86}$ G C A G A G G **G** C **G** T **G** **G** G G G A A A A G A A A $^{-63}$

TCE.m $^{-86}$ G C A G A G G **T** C **T** T **T** **T** G G G A A A A G A A A $^{-63}$

Figure 2. Sequence of the human c-*myc* promoter TGF-β1 control element (TCE) and mutant TCE (TCE.m). The sequence of the TCE in the c-*myc* promoter is shown compared to the sequence of TCE.m. Four transversion mutations in TCE create the mutant TCE.m. These constructs are used in Fig. 3.

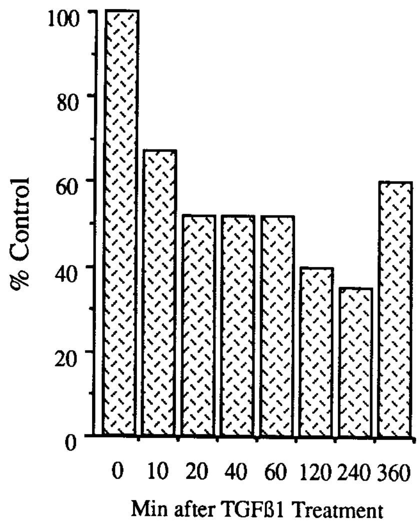

Figure 4. TGF-β1 regulation of TCE-protein binding in BALB/MK cells. Cellular proteins from rapidly growing BALB/MK cells treated with TGF-β1 (10 ng/ml) for the indicated times were separated by 7.5% SDS-PAGE, transferred to nitrocellulose filters, and incubated with 2.3×10^7 cpm of ^{32}P-labeled TCE hybrid. Binding was quantitated by laser scanning densitometry.

from SV40/HFK cells were performed to determine whether TCE bound to p106 in these cells and whether this binding was regulated by TGF-β1 treatment. The TCE probe did specifically bind to a 106-kD protein in the SV40/HFK cell extracts, and interestingly, the level of TCE-p106 binding was not affected by TGF-β1 treatment (data not shown).

To determine whether p106 could represent a modified form of pRB, extracts from various cell lines with a normal or altered *RB* gene were tested for TCE binding. These included DU145 cells, HFKs, PC3 (prostate adenocarcinoma cells containing a wild-type 105-kD *RB*) (Bookstein et al. 1990), and Y79 (retinoblastoma cells that have a deleted *RB* gene) (Whyte et al. 1988). All of the cell lines tested expressed a p106 protein that specifically bound the TCE, indicating that p106 is not a form of pRB (data not shown).

Finally, experiments were carried out to determine whether p106 could be found in complex with the adenovirus E1A, SV40 T antigen, and HPV-16 E7 oncoproteins. Antibodies that precipitate proteins associated with each of these oncoproteins were used to determine if TCE would specifically bind to the immunoprecipitated cellular protein complexes. However, TCE did not bind to any immunoprecipitated proteins, indicating that p106 is not in complexes with these viral oncoproteins (data not shown). This provides further evidence that p106 is not pRB and not the p107 cellular protein also found in complex with the viral oncoproteins.

DISCUSSION

Previous studies have implicated a cellular protein(s) that interacts with the pRB-binding domain of DNA tumor virus oncoproteins in the pathway for TGF-β1-induced suppression of c-*myc* transcription and cell proliferation in skin keratinocytes (Pietenpol et al. 1990b). Several cellular proteins, in addition to pRB, bind the conserved domain in the viral oncoproteins (DeCaprio et al. 1988; Whyte et al. 1988, 1989; Dyson et al. 1989; Giordano et al. 1989; Münger et al. 1989), and any of these proteins could be involved in suppression of c-*myc* transcription in response to TGF-β1. The RB protein was a good candidate, however, because stable transfection of the *RB* gene in a variety of cancer cell lines resulted in a suppression of tumorigenicity as well as a diminished growth potential of the cells (Huang et al. 1990; Bookstein et al. 1990). Furthermore, cotransfection of *RB* expression vectors with c-*myc* promoter reporter plasmids into skin keratinocytes inhibits c-*myc* expression in a manner analogous to TGF-β1 treatment (Pietenpol et al. 1991). The finding in the present study that TGF-β1 does not inhibit proliferation or reduce c-*myc* mRNA levels in *RB*-defective DU145 human prostatic adenocarcinoma cells is consistent with this hypothesis.

Evidence suggests that factors bind to the TCE; TCE formed three retarded complexes (TCE-I, TCE-II, and TCE-III) in gel mobility shift assays (Pietenpol et al. 1991). The TCE-II complex, but not the TCE-I or TCE-III complexes, was inhibited by TGF-β1 treatment of the TGF-β1-sensitive BALB/MK cells, but not the TGF-β1-insensitive HFK/SV40 and DU145 cells. It is not yet clear whether or not pRB is part of any of these complexes. No binding of the TCE oligonucleotide was obtained with cellular proteins coprecipitated with the DNA tumor virus oncoproteins or with antibodies to pRB. However, gel mobility shift data using extracts from cells that lack a functional pRB show prominent binding to TCE-II and TCE-III but a greatly diminished or absent TCE-I binding. Thus, it remains a possibility that the TCE-I complex could contain pRB as part of a multiprotein complex.

This paper describes our studies using Southwestern analysis to examine cellular protein binding to the TCE. Specific binding of the TCE to a protein of 106 kD (p106) was detected and this binding was regulated by TGF-β1 in TGF-β1-inhibited cells. The pattern of p106 suppression by TGF-β1 over time mirrored that of c-*myc* mRNA levels in keratinocytes treated with TGF-β1 (Coffey et al. 1988; Pietenpol et al. 1990a). Furthermore, p106 binding to TCE was not regulated by TGF-β1 in TGF-β1-insensitive cells (SV40/HFK cells). Binding was significantly decreased using a probe containing a mutated TCE (depicted in Fig. 2).

Kerr et al. (1990) have presented results indicating that TGF-β1 suppression of transin/stromelysin gene transcription is mediated through binding of a *fos*-containing protein complex to a promoter sequence, termed the *T*GF-β1 *i*nhibitory *e*lement (TIE). TIE also shows a region of sequence similarity to a region of the TCE (Pietenpol et al. 1991). A second region of sequence similarity to TCE within the human c-*myc* promoter is outside the 5′ region shown to be necessary for TGF-β1 suppression of c-*myc* transcription (Moses et al. 1990; Pietenpol et al. 1991). Robbins et al. (1990) have reported that expression of pRB in mouse fibroblasts suppresses transcription of c-*fos* and have identified an element, termed the retinoblastoma control element (RCE), in the c-*fos* promoter necessary for this suppression. More recently, sequences homologous to the RCE have been identified in the TGF-β1, TGF-β2, and TGF-β3 promoters by Kim et al. (1991). Cotransfection with an *RB* expression plasmid can regulate expression from TGF-β1 promoter/CAT constructs positively or negatively depending on the cell type (Kim et al. 1991). These investigators also reported that sequences similar to the RCE are present in the human c-*myc* promoter and that *RB* overexpression enhances c-*myc* expression in mink lung (Mv1Lu) cells, which are very sensitive to the growth inhibitory effect of TGF-β (Tucker et al. 1984). One of the sequences in the c-*myc* promoter they reported to be similar to the RCE is in the same region as TCE, but on the noncoding strand. This indicates that cell-type differences also exist and can influence the response pathway for TGF-β1 suppression of c-*myc* expression and growth inhibition.

Studies by Missero et al. (1991) have shown that transfection of keratinocytes with E1A renders the cells resistant to growth inhibition by TGF-β1. Mutational analysis indicated that these effects correlated with the ability of E1A proteins to bind pRB as well as other cellular proteins. Thus, it is possible that although pRB may be necessary for TGF-β1 suppression of c-*myc* transcription as indicated by our studies, inactivation of additional cellular proteins may be necessary to give full resistance to the growth inhibitory effects of TGF-β. Indeed, cellular proteins other than pRB are likely to be involved in the TGF-β1 suppression of c-*myc* transcription as indicated by the results of the binding studies presented here. Recent studies have indicated that the cellular transcription factor, E2F, associates with pRB (Bagchi et al. 1991; Chellappan et al. 1991; Chittenden et al. 1991), and it is possible that E2F may be one of the cellular factors capable of interaction with the TCE.

Previous experiments have revealed that pRB could inhibit c-*myc* expression in a manner similar to that seen by TGF-β1 treatment (Pietenpol et al. 1991). Because pRB can exist in various molecular-weight forms in SDS-PAGE due to a difference in pRB phosphorylation states (Whyte et al. 1988), the possibility that p106 was a form of pRB was examined. pRB-defective cell lines were examined by Southwestern analysis and were found to express a p106 protein that bound to the TCE probe. The possibility that p106 may be p107 (which binds the SV40 T antigen) (DeCaprio et al. 1988) or other proteins that bind adenovirus E2A (Yee and Branton 1985; Harlow et al. 1986; Giordano et al. 1989) or HPV E7 (Dyson et al. 1989; Münger et al. 1989) was also examined using antibodies to coprecipitate cellular factors in complex with the viral oncoproteins. TCE did not bind to the immunoprecipitated protein complexes.

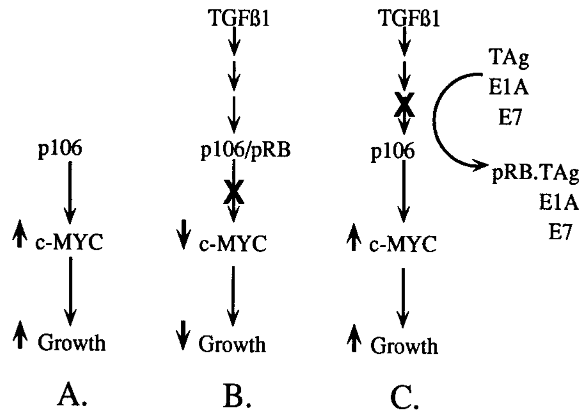

Figure 5. Schematic model of TGF-β1 inhibitory action on keratinocyte cell growth. This model represents possible modes of action for TGF-β1 inhibition of c-*myc* expression and subsequently, cell growth (see text).

The studies discussed here suggest that p106 may play a role in TGF-β1 suppression of c-*myc* and of keratinocyte growth. A model can be proposed to describe the mode of action of p106 (Fig. 5). TGF-β1 treatment suppresses TCE binding to p106, and this decreased binding correlates with TGF-β1 inhibition of c-*myc* transcription. These observations suggest that p106 may be a positive *trans*-activating factor for c-*myc* (Fig. 5A). Previous observations (Pietenpol et al. 1990b, 1991) and those reported here suggest that pRB is necessary for TGF-β1 suppression of c-*myc*. Since pRB apparently does not bind directly to TCE, it is possible that pRB binds p106 in response to TGF-β1 treatment. This results in decreased p106 binding to the TCE with resultant loss of *trans*-activation, as depicted in Figure 5B. However, there is as yet no direct evidence that this is the precise role for pRB in this pathway. The individual pRB-binding oncoproteins have been shown to confer TGF-β1 resistance on TGF-β1-sensitive keratinocytes (Pietenpol et al. 1990b). pRB may be inactivated or removed from this pathway by binding to these oncoproteins (Fig. 5C). This would allow p106 to *trans*-activate c-*myc* without TGF-β1 regulation. p106 does bind to the TCE in oncoprotein-immortalized SV40/HFK cells. Therefore, any of the *RB*-binding oncoproteins could release the keratinocytes from control by TGF-β1/*RB* and allow p106 to stimulate c-*myc* transcription and cell growth, causing the cells to become TGF-β1-resistant. Obviously, other models are possible, and further delineation of the exact sequence of events in this pathway will require cloning of the p106 gene and expression in appropriate systems to examine potential p106 binding to pRB and to show a direct effect of p106 on c-*myc* gene transcription.

SUMMARY

The TGF-βs are potent inhibitors of proliferation of most cell types in culture and in vivo. Previous studies have demonstrated that TGF-β inhibition of skin keratinocyte proliferation involves suppression of c-*myc* transcription. Evidence derived from use of expression plasmids for certain DNA viral oncoproteins has suggested that the retinoblastoma gene (*RB*) may be involved in this process. Transient expression of pRB, like TGF-β1, in skin keratinocytes represses expression of a human c-*myc* reporter plasmid, and the same c-*myc* promoter region (TCE) is required for repression by either TGF-β1 or pRB. We showed here that proliferation and c-*myc* expression in a cell line lacking normal pRB (DU145 human prostate adenocarcinoma cells) are not inhibited by TGF-β1. Oligonucleotides containing the TCE were found to bind to a cellular protein of approximately 106 kD (termed p106) in Southwestern assays, utilizing extracts from both the skin keratinocytes and DU145 cells. TCE binding to p106 was diminished by TGF-β in TGF-β-sensitive skin keratinocytes but not in TGF-β-insensitive SV40-transformed keratinocytes. These data support the hypothesis that pRB is required for TGF-β1 suppression of c-*myc* transcription and suggest the involvement of a cellular factor(s) in addition to pRB in the TGF-β1 pathway for inhibition of c-*myc* transcription and growth inhibition.

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